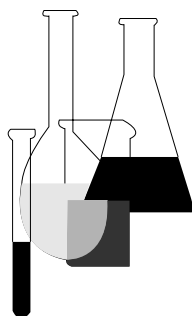




Health Effects Test Guidelines OPPTS 870.1300 Acute Inhalation Toxicity



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on disks or paper copies: call (202) 512-0132. This guideline is also available electronically in PDF (portable document format) from EPA's World Wide Web site (<http://www.epa.gov/epahome/research.htm>) under the heading "Researchers and Scientists/Test Methods and Guidelines/OPPTS Harmonized Test Guidelines."

OPPTS 870.1300 Acute inhalation toxicity.

(a) **Scope—(1) Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source materials used in developing this harmonized OPPTS test guideline are 40 CFR 798.1150 Acute Inhalation Toxicity; OPP 81–3 Acute Inhalation Toxicity-Rat(Pesticide Assessment Guidelines, Subdivision F—Hazard Evaluation; Human and Domestic Animals) EPA report 540/09–82–025, 1982; and OECD guideline 403 Acute Inhalation Toxicity.

(b) **Purpose.** Determination of acute toxicity is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance that may be inhaled such as a gas, volatile substance, or aerosol/particle. It provides information on health hazards likely to arise from short-term exposure by the inhalation route. Data from an acute study may serve as a basis for classification and labeling. It is traditionally a step in establishing a dosage regimen in subchronic and other studies and may provide initial information on the mode of toxic action of a substance. An evaluation of acute toxicity data should include the relationship, if any, between the animals' exposure to the test substance and the incidence and severity of all abnormalities, including behavioral and clinical abnormalities, the reversibility of observed abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects.

(c) **Definitions.** The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in 40 CFR Part 792—Good Laboratory Practice Standards apply to this test guideline. The following definitions also apply to this test guideline.

Acute inhalation toxicity is the adverse effect caused by a substance following a single uninterrupted exposure by inhalation over a short period of time (24 h or less) to a substance capable of being inhaled.

Aerodynamic equivalent diameter is defined as the diameter of a unit-density sphere having the same terminal settling velocity as the particle in question, whatever its size, shape, and density. It is used to predict where in the respiratory tract such particles may be deposited.

Concentration is expressed as weight of the test substance per unit volume of air, e.g. milligrams per liter.

Inhalable diameter refers to that aerodynamic diameter of a particle which is considered to be inhalable for the organism under study. It is used to refer to particles which are capable of being inhaled and deposited anywhere within the respiratory tract .

LC_{50} (median lethal concentration) is a statistically derived estimate of a concentration of a substance that can be expected to cause death during exposure or within a fixed time after exposure in 50 percent of animals exposed for a specified time. The LC_{50} value is expressed as weight of test substance per unit volume of air (milligrams per liter) or parts per million. For clarity, the exposure duration should also be specified, e.g. 4-h LC_{50} .

Mass median aerodynamic diameter (MMAD) is the median aerodynamic diameter and, along with the geometric standard deviation, is used to describe the particle size distribution of any aerosol statistically, based on the weight and size of the particles. Fifty percent of the particles by weight will be smaller than the median diameter and 50 percent of the particles will be larger.

(d) Approaches to the determination of acute toxicity. (1) EPA recommends the following means to reduce the number of animals used to evaluate acute effects of chemical exposure while preserving its ability to make reasonable judgments about safety:

(i) Using data from substantially similar mixtures. In order to minimize the need for animal testing, the Agency encourages the review of existing acute toxicity information on mixtures that are substantially similar to mixtures under investigation. In certain cases, it may be possible to get enough information to make preliminary hazard evaluations that may reduce the need for further animal testing.

(ii) Limit test. When data on structurally related chemicals are inadequate, a limit test may be considered. In the limit test, a single group of five males and five females is exposed to 2 mg/L for 4 h, or where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration, using the procedures described under paragraph (e) of this guideline. If no lethality is demonstrated, no further testing for acute inhalation toxicity is needed. If compound-related mortality is produced, further study may need to be considered.

(2) [Reserved]

(e) Conventional acute toxicity test—(1) Principle of the test method. Several groups of experimental animals are exposed to the test substance in graduated concentrations for a defined period, one concentration being used per group. When a vehicle other than water is used to help generate an appropriate concentration of the substance in the atmosphere, a vehicle control group should be used when historical data are not available or adequate to determine the acute inhalation toxicity of the vehicle. Subsequently, observations of effects and death are made. Animals that die during the test are necropsied and at the conclusion of the test surviving animals are sacrificed and necropsied. This guideline is directed pri-

marily to studies in rodent species but may be adapted for studies in non-rodents. Animals showing severe and enduring signs of distress and pain may need to be humanely killed. Dosing test substances in a way known to cause marked pain and distress due to corrosive or irritating properties need not be carried out.

(2) **Substance to be tested.** Test, control, and reference substances are discussed in 40 CFR part 792, subpart F (Good Laboratory Practice Standards).

(3) **Test procedure**—(i) **Preparation.** Healthy young adult animals are acclimatized to the laboratory conditions for at least 5 days prior to the test. Before the test, animals are randomized and assigned to the required number of groups.

(ii) **Animal selection**—(A) **Species and strain.** Although several mammalian test species may be used, the preferred species is the rat. Commonly used laboratory strains should be employed. If another mammalian species is used, the tester should provide justification and reasoning for its selection.

(B) **Age.** Young adult rats between 8–12 weeks old at the beginning of dosing, should be used. The weight variation in animals or between groups used in a test should not exceed ± 20 percent of the mean weight of each sex.

(C) **Number and sex.** (1) At least five experimentally naive animals are used at each concentration and they should be of one sex. After completion of the study in one sex, at least one group of five animals of the other sex is exposed to establish that animals of this sex are not markedly more sensitive to the test substance. The use of fewer animals may be justified in individual circumstances. Where adequate information is available to demonstrate that animals of the sex tested are markedly more sensitive, testing in animals of the other sex is not required. An acceptable option would be to test at least one group of five animals per sex at one or more dose levels to definitively determine the more sensitive sex prior to conducting the main study.

(2) Females should be nulliparous and nonpregnant.

(3) In acute toxicity tests with animals of a higher order than rodents, the use of smaller numbers should be considered.

(D) **Assignment of animals.** Each animal must be assigned a unique identification number. A system to assign animals to test groups and control groups randomly is required.

(E) **Housing.** The animals may be group-caged by sex, but the number of animals per cage must not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (e.g.

morbidity, excitability) may indicate a need for individual caging. Animals must be housed individually in inhalation chambers during exposure to aerosols.

(1) Before and after exposure, the temperature of the animal room should be 22 ± 3 °C and the relative humidity 30–70 percent.

(2) Where lighting is artificial, the sequence should be 12 h light/12 h dark.

(3) For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water.

(F) **Equipment.** (1) The animals should be tested with inhalation equipment designed to sustain a dynamic air flow of at least 10 air changes per hour, an adequate oxygen content of at least 19 percent, and uniform conditions throughout the exposure chamber. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas. It is normally not necessary to measure chamber oxygen concentration if airflow is adequate.

(2) The selection of a dynamic inhalation chamber should be appropriate for the test article and test system. Where a whole body chamber is used to expose animals to an aerosol, individual housing must be used to minimize crowding of the test animals and maximize their exposure to the test substance. To ensure stability of a chamber atmosphere, the total volume of the test animals should not exceed 5 percent of the volume of the test chamber. It is recommended, but not required, that nose-only or head-only exposure be used for aerosol studies in order to minimize oral exposures due to animals licking compound off their fur. The animals should be acclimated and heat stress minimized.

(G) **Physical measurements.** Measurements or monitoring should be made of the following:

(1) The rate of air flow should be monitored continuously, but recorded at least 3 times during the exposure.

(2) The actual concentrations of the test substance should be measured in the breathing zone. During the exposure period, the actual concentrations of the test substance shall be held as constant as practicable and monitored continuously or intermittently depending on the method of analysis. Chamber concentration may be measured using gravimetric or analytical methods as appropriate. If trial run measurements are reasonably consistent (± 10 percent for liquid aerosol, gas, or vapor; ± 20 percent for dry aerosol), then two measurements should be sufficient. If measurements are not consistent, three to four measurements should be taken. Whenever the test article is a formulation, the analytical concentration must be reported for the total formulation, and not just for the active ingredient (AI). If,

for example, a formulation contains 10 percent AI and 90 percent inerts, a chamber analytical limit concentration of 2 mg/L would consist of 0.2 mg/L of the AI. It is not necessary to analyze inert ingredients provided the mixture at the animal's breathing zone is analogous to the formulation; the grounds for this conclusion must be provided in the study report. If there is some difficulty in measuring chamber analytical concentration due to precipitation, nonhomogeneous mixtures, volatile components, or other factors, additional analyses of inert components may be necessary.

(3) During the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations. The MMAD particle size range should be between 1–4 μm . The particle size of hygroscopic materials should be small enough when dry to assure that the size of the swollen particle will still be within the 1–4 μm range. Measurements of aerodynamic particle size in the animal's breathing zone should be measured during a trial run. If MMAD values for each exposure level are within 10 percent of each other, then two measurements during the exposures should be sufficient. If pretest measurements are not within 10 percent of each other, three to four measurements should be taken.

(4) Temperature and humidity should be monitored continuously and recorded at least 3 times during exposure. The temperature at which the test is performed should be maintained at 22 ± 2 °C. The relative humidity should be maintained between 30 and 70 percent humidity, but in certain instances (tests of aerosols) this may not be practicable.

(iii) **Exposure duration and levels.** (A) Shortly before exposure, the animals are weighed and then exposed to the test concentration in the designated apparatus for 4 h after equilibration of the chamber concentrations. Other durations may be needed to meet specific requirements. Food should be withheld during exposure. Water may also be withheld in certain circumstances.

(B) **Exposure levels.** Three concentration levels should be used and spaced appropriately to produce a concentration-response curve and permit an estimation of the median lethal concentration. Range-finding studies using single animals may help to estimate the positioning of the test groups so that no more than three concentration levels will be necessary. An acceptable option for pesticide products would be to set the dose levels in correlation with the OPP toxicity categories (bracketing). In these cases, the determination of an LD_{50} may not be necessary.

(iv) **Observation period.** The observation period should be at least 14 days. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset, and length of recovery period, and thus may be extended when considered necessary. The time at which signs of toxicity appear, their duration, and the time

of death are important, especially if there is a tendency for deaths to be delayed.

(v) **Observation of animals.** (A) A careful clinical examination should be made at least once each day.

(B) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study, e.g. necropsy or refrigeration of those animals found dead and isolation of weak or moribund animals.

(C) Observations should be detailed and carefully recorded, preferably using explicitly defined scales. Observations should include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypies or bizarre behavior (e.g., self mutilation, walking backwards).

(D) Individual weights of animals should be determined prior to exposure, weekly after exposure, and at death. Changes in weights should be calculated and recorded when survival exceeds 1 day.

(E) The time of death should be recorded as precisely as possible.

(vi) **Gross pathology.** (A) At the end of the test, surviving animals should be weighed and sacrificed.

(B) A gross necropsy should be performed on all animals under test, with particular reference to any changes in the respiratory tract. All gross pathology changes should be recorded.

(C) If necropsy cannot be performed immediately after a dead animal is discovered, the animal should be refrigerated (not frozen) at temperatures low enough to minimize autolysis. Necropsies should be performed as soon as possible, normally within a day or two.

(viii) **Additional evaluations.** In animals surviving 24 h or more, microscopic examination of organs showing evidence of gross pathology should be considered since it may yield useful information.

(ix) **Data and reporting—(A) Treatment of results.** Data should be summarized in tabular form showing for each test group the number of animals at the start of the test, body weights, time of death of individual animals at different exposure levels, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings. Any method used for calculation of the LC_{50} or any other parameters should be specified and referenced. Methods for parameter estimation are described under paragraphs (f)(1), (f)(2), and (f)(3) of this guideline.

(B) Evaluation of results. The LC₅₀ value should be considered in conjunction with the observed toxic effects and the necropsy findings. The LC₅₀ value is a relatively coarse measurement useful only for classification and labeling purposes and an expression of the lethal potential of the test substance following inhalation. Reference should always be made to the experimental animal species and exposure duration in which the LC₅₀ value was obtained. An evaluation should include the relationship, if any, between exposure of animals to the test substance and the incidence and severity of all abnormalities including behavioral and clinical abnormalities, gross lesions, body weight changes, mortality, and other toxic effects.

(C) Test report. In addition to the reporting requirements as specified under 40 CFR part 792, subpart J and 40 CFR 160, subpart J, the following specific information should be reported.

(1) Test conditions. (i) Description of exposure apparatus including design, type, dimensions.

(ii) Source of air, system for generating the test article.

(iii) Equipment for measuring temperature, humidity, particle size, and actual concentration.

(iv) Treatment of exhaust air and the method of housing the animals in a test chamber when this is used.

(2) Exposure data. These should be tabulated and presented with mean values and a measure of variability (e.g. standard deviation) and should include:

(i) Airflow rates through the inhalation equipment.

(ii) Temperature and humidity of the air.

(iii) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(iv) Actual (analytical or gravimetric) concentration in test breathing zone.

(v) Particle size distribution, and calculated MMAD and geometric standard deviation (GSD).

(vi) Explanation as to why the desired chamber concentration and/or particle size could not be achieved (if applicable), and the efforts taken to comply with these aspects of the guidelines.

(3) Species, strain, sex, and source of test animals.

(4) Method of randomization in assigning animals to test and control groups.

(5) Rationale for selection of species, if other than that recommended.

(6) Tabulation of individual and test group data by sex and exposure level (e.g. number of animals exposed, number of animals showing signs of toxicity and number of animals that died or were killed during the test).

(i) Description of toxic effects including their time of onset, duration, reversibility, and relationship to concentration.

(ii) Body weights.

(iii) Time of dosing and time of death during or following exposure.

(iv) Concentration-response curves for mortality and other toxic effects (when permitted by the method of determination).

(v) Gross pathology findings.

(vi) Histopathology findings and any additional clinical chemistry evaluations if performed.

(7) Description of any pretest conditioning, including diet, quarantine and treatment for disease.

(8) Description of caging conditions, including: number (or change in number) of animals per cage, bedding material, ambient temperature and humidity, photoperiod, and identification of diet of test animals.

(9) Manufacturer (source), lot number, and purity of test substance.

(10) Identification and composition of any vehicles (e.g., diluents, suspending agents, and emulsifiers) or other materials used in administering the test substance.

(11) A list of references cited in the body of the report. References to any published literature used in developing the test protocol, performing the testing, making and interpreting observations, and compiling and evaluating the results.

(f) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Chanter, D.O. and Heywood, R. The LD₅₀ test: some considerations of precision. *Toxicology Letters* 10:303–307 (1982).

(2) Finney, D.G. Chapter 3—Estimation of the median effective dose, Chapter 4—Maximum likelihood estimation. *Probit Analysis*. 3rd Ed. (Cambridge, London. (1971).

(3) Finney, D.J. The Median Lethal Dose and Its Estimation, *Archives of Toxicology* 56:215–218 (1985).

(4) Organization for Economic Cooperation and Development. OECD Guidelines for the Testing of Chemicals. Final Draft OECD Guideline 425: Acute Oral Toxicity: Up-and-Down Procedure to be adopted in the Tenth Addendum to the OECD Guidelines for the Testing of Chemicals.

(5) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 403: Acute Inhalation Toxicity. Adopted: May 12, 1981.

(6) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 420: Acute Oral Toxicity—Fixed Dose Method. Adopted: July 17, 1992.

(7) Organization for Economic Cooperation and Development. OECD Guidelines for Testing of Chemicals. Guideline 423: Acute Oral Toxicity—Acute Toxic Class Method. Adopted: March 22, 1996.

(8) U. S. EPA. Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies. 2/1/94. Health Effects Division, Office of Pesticide Programs.